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Comparative analysis of histological results of visible lesion thresholds for thermal and LIB induced skin damage at 1.3 μm and 1.5 μm

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ABSTRACT

An assessment of skin damage caused by near-IR laser exposures is reported. The damage from two distinct laser-tissue temporal regimes is compared at two wavelengths (1.3 μm and 1.5 μm). Skin damage caused by thermal effects from single laser pulses is compared to damage caused by LIB (laser induced breakdown) using histological examinations. Modeling applications are explored to determine crossover points between thermal and photomechanical damage thresholds.

Keywords: laser, histopathology, skin, near-IR, MVL, safety, LIB

I. INTRODUCTION

Recent technical developments in solid state and chemical lasers have facilitated the growing use of pulsed, high-peak-power lasers at the 1300-nm and 1500-nm wavelengths. Numerous medical, military and industrial applications have been identified. While several ocular injury studies for 1300 and 1500 nm exist, and the interaction between the components of the eye and NIR laser radiation is fairly well understood, few skin injury studies have been conducted at these wavelengths, especially for Q-switched laser operation. Prior to the recent advancements in the capability of these laser systems, particularly the 1540-nm regime, little concern was given to potential skin hazards associated with these systems. However, higher-energy systems at these wavelengths are becoming available in smaller, more reliable packages. Table-top laser systems are capable of producing microsecond pulses with tens of joules of energy per pulse.¹

Once the first experimental data showed the benefits of picosecond and femtosecond pulses compared to nanosecond pulses for ablation-mediated, precise tissue removal in fairly transparent tissue², few studies have explored the damage mechanism of surface LIB plasma damage on skin from nanosecond pulses in the NIR. Due to the proliferation of high-peak-power systems in the NIR for military and industrial applications, the damage mechanisms associated with these systems are now of interest from a laser-safety perspective. Recent studies^{3,4} using high-peak-power lasers at the 1300-nm and 1500-nm wavelengths have shown that current thermal damage models are insufficient to predict damage when single pulses in the nanosecond regime are considered. To gain insight as to the damage mechanisms present at these temporal and energy regimes, a comparative histological analysis has been conducted for tissue samples exposed to three laser wavelengths and pulse conditions. The three laser exposure conditions explored in this work are single-pulse exposures at 1540 nm with pulse durations of 600 μs and 35 ns, and 1318 nm with a pulse duration of 50 ns. All three exposures were delivered with a 5-mm beam diameter and flat-top profile. The subject models in all three exposures were Yucatan mini-pig (*Sus scrofa domestica*). The histological

samples were gathered using 5-mm punch biopsies taken at 1 hour and 24 hours post exposure where gross lesions were observed.

II. MATERIALS AND METHODS

The study progressed under the animal use protocol titled “Evaluation of Laser induced Corneal Lesions in the Dutch Belted Rabbit and Skin Lesions in the Yucatan Mini-Pig,” which was approved by the Brooks City-Base, TX Institutional Animal Care and Use Committee (IACUC). None of the animals were euthanized after exposure or biopsy. The animals involved in this study were procured, maintained, and used in accordance with the Federal Animal Welfare Act and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources -- National Research Council. Brooks City-Base, TX has been fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) since 1967.

2.1 Laser exposures & ED₅₀ determination

The exposures for the determination of the dose which has a 50% probability of producing a lesion, referred to as the ED₅₀, at 1318 nm were delivered using a Nd:YAG, Q-switched laser. The laser produced a single pulse at 50-ns duration. The delivered energy of a single pulse ranged from ~100 mJ to 3 J. A flashlamp-pumped, Er:Glass laser was used to provide the exposures at 1540 nm. The laser produced pulses in the 30–40-ns range at various pulse energies up to 3.5 joules per pulse

During the exposures, it was noted if an exposure caused an audible “pop”, a visible flash, or both. The exposed skin sites were examined at 1 hour and 24 hours post exposure. Three trained personnel independently examined each exposure site and recorded either “lesion” or “no lesion” for each site. Once the three examinations were finished, the individual results were compared. Agreement of at least two of the three readers was needed for a positive lesion result to be confirmed.

The Probit⁵ statistical analysis package was used to calculate the ED₅₀ for each wavelength. Supporting the analyses of the ED₅₀, the fiducial limit at the 95% confidence level, along with the slope and probability of the Probit curve was also calculated at each wavelength at both 1 hour and 24 hours post exposure. The Probit analysis results for the three studies which are histologically evaluated are presented in Table 1.

Table 1: 1-hr and 24-hr ED₅₀ for laser exposures to porcine skin. Fiducial limits are shown in parentheses.

Laser Parameters Number of Subjects & Exposures	ED ₅₀ (J cm ⁻²) 1-Hour Reading	ED ₅₀ (J cm ⁻²) 24-Hour Reading
1318 nm 5-mm beam diameter 50 ns ⁴	6.5 (7.0 – 5.9)	10.5 (11.1 – 10.0)
1540 nm 5-mm beam diameter 30 ns ⁴	6.3 (6.8 – 5.8)	6.1 (6.5 – 5.5)
1540 nm 5-mm beam diameter 600 µs ³	6.4 (6.7 – 6.2)	7.4 (7.8 – 7.0)

2.2 Histopathologic examinations

Five-millimeter diameter, full-thickness skin biopsies were fixed in 10% neutral buffered formalin and submitted for routine histopathologic examination. The biopsies were bisected, routinely processed, and embedded in paraffin blocks. Five-micron thick sections were cut, mounted on positively charged glass slides, and stained with hematoxylin and eosin (HE).

III. HISTOLOGICAL FINDINGS

3.1 1540-nm, 600- μ s exposures

Generally, all the lesions observed in the 1540-nm exposures are characterized by a conical-shaped area of coagulative necrosis with the apex extending into the superficial dermis. The lesions caused by the 1540-nm wavelength, 600- μ s pulse duration, 5.12-J/cm² radiant exposure insult extended into the dermis to a depth of 0.4 mm; while the 1540-nm wavelength, 600- μ s pulse duration, 9.72-J/cm² radiant exposure lesion extended into the dermis to a depth of 1.0 mm. The two lesions are shown side-by-side in Figure 1. The depth of the lesion into the dermis is directly proportional to the radiant exposure. The affected epidermis is condensed, hypereosinophilic and characterized by epithelial cells that exhibit a loss of differential staining and loss of cellular detail with retention of the basic epidermal architecture, nuclear pyknosis, and karyolysis. Multifocally, the epidermis is separated from the dermis creating variably sized clefts. There is edema fluid filling the dermoepidermal clefts in the 9.72 J/cm² radiant exposure. The affected collagen has an amorphous coagulated appearance characterized by a loss of the fibrillar architecture and pale basophilic staining.

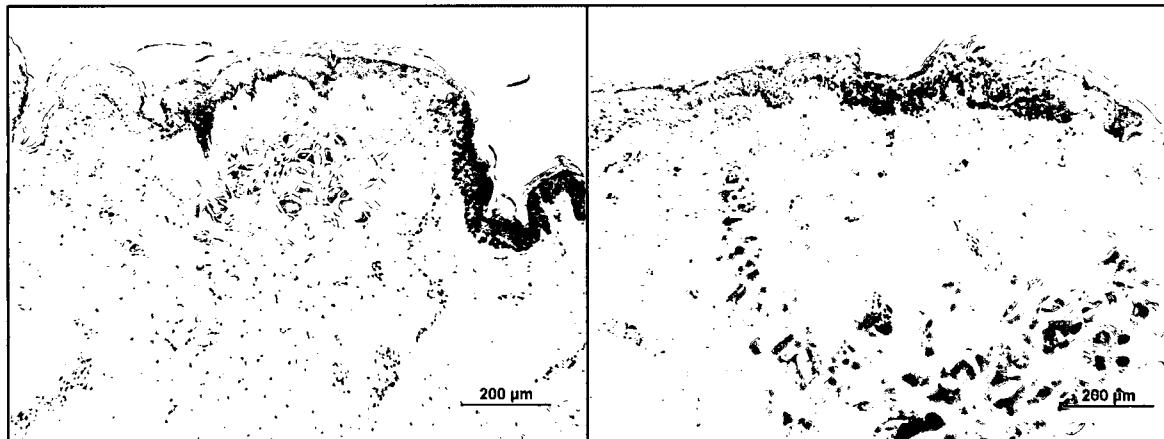


Figure 1: *LEFT:* H&E stained biopsy, 100x magnification, of laser-exposed porcine skin with laser parameters of 1540 nm, 600- μ s pulse duration, and a radiant exposure of 5.12 J/cm² taken 24 hours post exposure. Sample shows a second-degree burn. There is a conical, 0.4-mm deep area of coagulative necrosis affecting the epidermis and extending into the superficial dermis.

RIGHT: H&E stained biopsy, 100x magnification, of laser-exposed porcine skin with laser parameters of 1540 nm, 600- μ s pulse duration, and a radiant exposure of 9.72 J/cm² taken 24 hours post exposure. Sample shows a second-degree burn. There is a conical, 1.0-mm deep area of coagulative necrosis affecting the epidermis and extending into the superficial dermis.

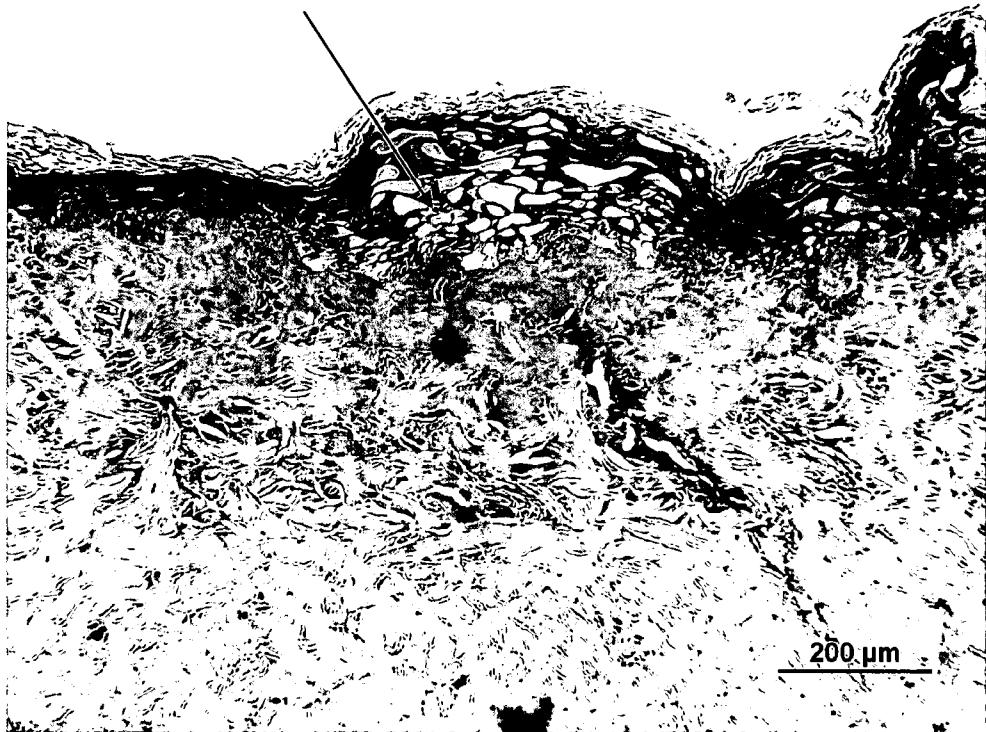


Figure 2: H&E stained biopsy, 100x magnification, of laser exposed porcine skin with laser parameters of 1540 nm, 35-ns pulse duration, and a radiant exposure of 6.38 J/cm^2 taken 1 hour post exposure. The sample shows effects of a second-degree burn. Coagulative necrosis of the epidermis and superficial dermis with microvesicular disruption of the epithelium can be seen (arrow).

3.2 1540-nm, 35-ns exposures

The lesion present in the 1540-nm wavelength, 35-ns pulse duration laser exposure with a radiant exposure of 8.03 J/cm^2 , is a second-degree burn extending 0.5 mm into the dermis, and is characterized by coagulative necrosis of the epidermis and superficial dermis as described above. However, this lesion differs from the 600- μs pulse duration exposure in that centrally there is marked microvesicular disruption of the epidermis, as seen in Figure 2. The variably sized and coalescing vesicles contain an eosinophilic to lightly basophilic material interpreted as edema fluid.

3.3 1318-nm, 50-ns exposures

Histopathologic examination of a 1318-nm, 50-ns pulse duration, 10.24-J/cm^2 radiant exposure laser insult, taken by biopsy 1 hour post exposure, reveals epithelial cell degeneration and coagulative necrosis of the middle and basal layers of the epidermis. The lesion is concentrated on the pigmented epithelial cells, sparing the superficial epidermal layers as seen in Figure 3. All of the 1318-nm, 50-ns exposures exhibited similar damage patterns, with the damage limited to the cell layers which contain melanin. The degenerate and necrotic cells are characterized by a loss of differential staining and cellular detail with retention of the basic epidermal architecture, hydropic degeneration, nuclear pyknosis, and karyolysis. There is mild congestion of the vascular plexus in the superficial dermis.



Figure 3: H&E stained biopsy, 100x magnification, of laser exposed porcine skin with laser parameters of 1318 nm, 50-ns pulse duration, and a radiant exposure of 10.24 J/cm^2 taken 1 hour post exposure. Epithelial cell degeneration and coagulative necrosis of the middle and basal layers of the epidermis is observed. Note that the damage is confined to the cells layers where pigmented melanin is present.

IV. DISCUSSION

4.1 Comparison between 1540-nm, 600-μs pulses and 1540-nm, 35-ns pulses

The histological results of the three laser exposure conditions aid in identifying the damage mechanism present. Several trends are noticeable from the histological analysis of the irradiated tissue samples. The comparison between the 1540-nm, 600- μs exposures and the 1540-nm, 35-ns exposures are quite interesting. The difference in pulse duration is nearly four orders of magnitude and it is this parameter that determines which damage mechanism dominates. The longer pulse duration exposures are within thermal confinement⁶ and show many of the signs of thermal injury. From the histological findings, the penetration depth of the injury correlates well with the radiant exposure. In the sample on the left in Figure 1, the injury extends 0.4 mm into the tissue. This lesion was caused by an exposure of 5.12 J/cm^2 . The injury shown on the right in Figure 1 extends nearly 1.0 mm into the tissue and was caused by a radiant exposure of 9.72 J/cm^2 . Both of these lesions were 24 hours post exposure.

The damage caused by the 35-ns exposures at 1540 nm (Figure 2) look fundamentally different compared to the lesions caused by the 600- μs pulse at 1540 nm (Figure 1). These findings are consistent with the understanding of laser-tissue interactions in the nanosecond regime. The pulse parameters place it in the stress confinement regime, and the peak irradiance associated with the ED₅₀ is sufficient to produce an LIB (laser induced breakdown) event at the air tissue surface interface.⁷ The pooling of fluid present at epidermal layer may be caused by the effects of plasma formation at the surface of the skin. The formation of the plasma can produce a mechanical shockwave caused by the rapidly expanding plasma cloud and the extremely high pressures associated with its formation. Temperatures associated with plasma formation

can reach several thousand degrees K.⁸ The large blistering areas of the epidermal layer could have been caused by a combination of effects attributed to the formation of a plasma. The mechanical shockwave compresses and stretches the biological medium it traverses. These stresses may make the tissue more susceptible to the heat generated by the plasma cloud.

4.2 Comparison between 1318-nm, 50-ns pulses and 1540-nm, 35-ns pulses

While the comparison between the microsecond, thermal regime and the nanosecond, plasma mediated damage regime is interesting, curious results between nanosecond pulses of different wavelengths has been observed.

The most interesting difference between the two wavelengths is the change in the 24-hour post exposure minimal visible lesion (MVL) ED₅₀ levels. The 1540-nm exposure ED₅₀ level remains essentially constant from 1 hour to 24 hours post exposure, whereas the 1318-nm exposures showed an increase in the ED₅₀ level of nearly two-fold from 1 hour to 24 hours post exposure. The source of this difference is not completely understood. As seen in Table 2, both water and the dermal and epidermal skin layers exhibit different absorption coefficients (μ_a) at the two wavelengths. This could be the basis the discrepancy between the 1 hour and 24 hour ED₅₀ levels for the two wavelengths. For nanosecond pulse durations, the optical breakdown threshold is determined by laser heating of absorbing impurities at the surface of irradiated medium and associated thermal losses.² Since the absorption properties are different at each wavelength, a possible explanation for the difference in irradiance could be due to how efficiently plasma is created at the different wavelengths. If more of the laser energy at 1318 nm is required to raise the temperature of the surface to the point of plasma formation, less of the laser energy would be available for sustaining the plasma growth. This would lead to less intense plasma clouds with shorter durations produced by the 1318-nm laser exposures. There is some qualitative, anecdotal evidence that supports this idea. The plasmas produced by the 1540-nm laser exposures were noted to be more readily visible and accompanied by a louder and sharper acoustic “pop.” There are also some histological findings that would agree with the assumption that some 1318-nm laser energy is penetrating into the tissue. One would not expect a mechanical pressure wave to be selective as to which cells are damaged. Also the thermal energy created by the plasma is unlikely to penetrate that deeply into the tissue. It appears that the higher absorption coefficient of melanin at 1318 nm plays the dominant role in causing this damage as noted by Jacques.⁹ This supports the idea that more 1318-nm laser radiation is transmitted into the tissue and it takes a higher-energy exposure to cause damage that persists for 24 hours post exposure.

Table 2: Absorption coefficients for skin layers and water at 1315 and 1540 nm.

Medium	λ (nm)	μ_a (cm ⁻¹)	Ratio of μ_a (1540 nm) to μ_a (1315 nm)
Epidermis	1315*	1.07	5.6
	1540*	6.0	
Dermis	1315*	1.19	4.55
	1540*	5.42	
Water	1315†	1.79	7.62
	1540†	13.0	

* Measurements for this study made by Welch's group at UT-Austin, † Reference 10.

4.3 Absorption coefficient and LIB threshold

Looking at the literature, a relationship between absorption coefficients and LIB and damage thresholds, assuming that the damage threshold for nanosecond pulses coincides with the LIB threshold, for biological and surrogate tissues can be seen. In Figure 4, a plot of selected LIB/damage thresholds vs. absorption coefficients for skin, cornea and surrogate tissues. The data represent pulse durations from 1 to 100 ns, spot sizes from 0.5 to 10 mm, and wavelengths from 1.06 μm to 3.9 μm . The nanosecond pulse duration exposures evaluated in this work fit well with the rest of the data. A description of each point is given in Table 3. The dependency of damage thresholds and absorption coefficients supports the explanations for the difference in ED_{50s} and the histological evaluations given. This relationship does not extend to the pico and femtosecond pulse duration where non-linear effects govern the formation of LIB.

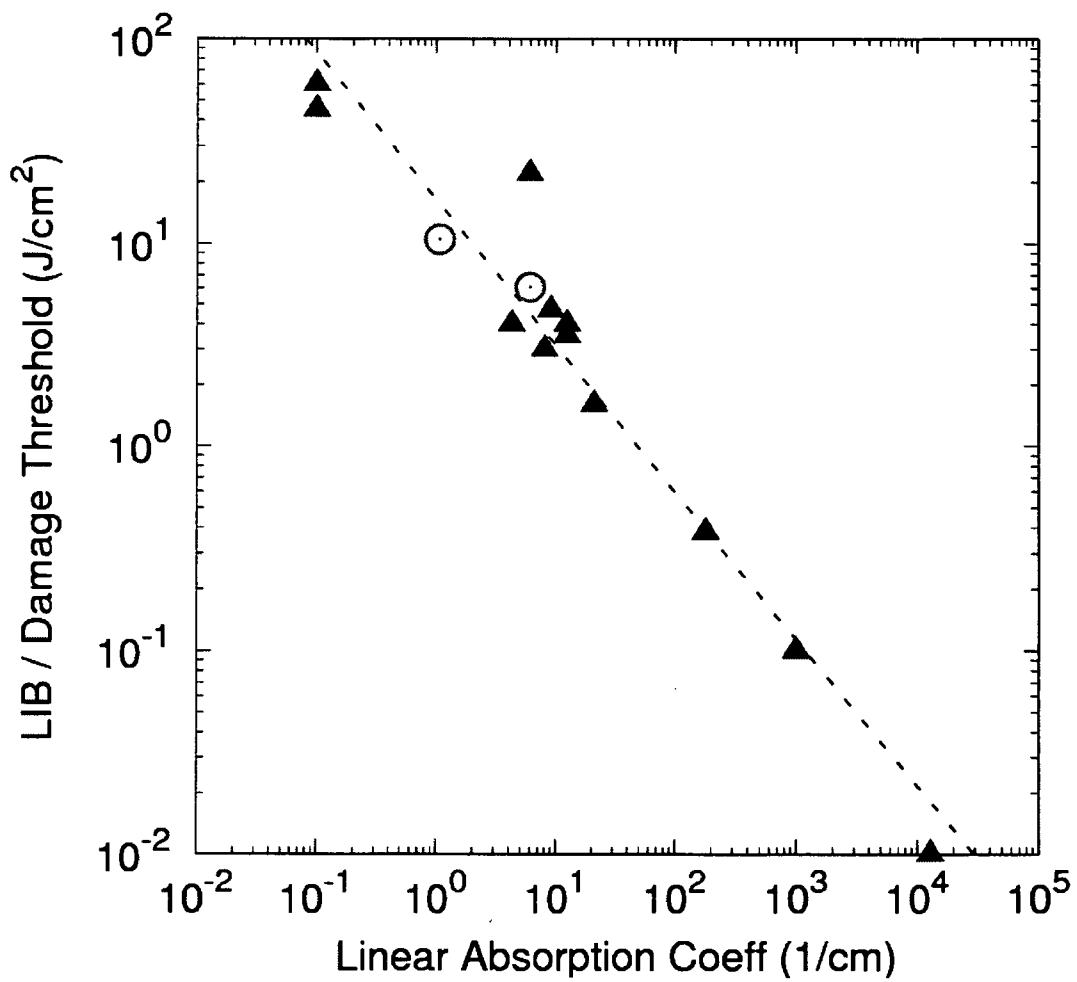


Figure 4: LIB damage thresholds vs. absorption coefficients for laser wavelengths in the NIR and far-IR on skin and corneal tissue. The data points represented with red circles are the Q-switched exposures examined in this work.

Table 3: Selected ED₅₀/LIB threshold data from the literature. The data are plotted in Figure 4.

Medium	λ (μm)	Pulse duration (ns)	Spot size (mm)	μ_a (cm^{-1})	ED ₅₀ /LIB Threshold (J/cm ²)	Ref.
Porcine cornea	1.053	1	0.5	0.1	60	2
Clear collagen gel	1.053	1	0.5	0.1	45	2
Collagen gel colored with cupric sulfate	1.053	1	0.5	22	6.0	2
Collagen gel with carbon black micropowder	1.053	1	0.5	1000	0.01	2
Porcine skin (Yucatan mini-pig)	1.318	50	5	1.1	10.5	4
Pigmented ballistic media	1.54	35	5	20	1.3	11
Guinea pig skin	1.54	35	5	8	3.0	12
Porcine skin (Yucatan mini-pig)	1.54	35	5	6	6.1	4
Porcine skin (Yorkshire)	1.54	100	4.2	12.4*	4	13
Porcine skin (Yorkshire)	1.54	100	2.6	12.4*	3.5	13
Primate cornea	1.54	40	1	9.03	4.7	14
Primate cornea	2.9	100	10	12900*	0.008	15
Primate cornea	3.6-3.9	100	1	150*	0.377	16

* Absorption coefficients estimated using absorption coefficient for water.

V. CONCLUSIONS

Once benefits of picosecond and femtosecond pulses over nanosecond pulses for ablation-mediated, precise tissue removal were found, few studies have explored the damage mechanism of surface LIB plasma damage on skin from nanosecond pulses¹⁷ particularly for millimeter spot sizes. Histological evaluations are reported for the wavelengths of 1318 nm and 1540 nm with pulse durations of 50 ns and 35 ns, respectively; as well as exposures at 1540 nm with pulse durations of 600 μ s. Comparisons between the histological evaluations and the ED₅₀ levels of the nanosecond pulse duration exposures are made. Bulk media absorption properties have been considered to be the driving factor in thresholds for LIB in the nanosecond regime⁶ and data associated with this study and the literature confirm this. Data from the literature are presented which ranges from 1 ns to 100 ns pulse duration with absorption coefficients spanning 5 orders of magnitude.

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REFERENCES

1. Taboada, J., J.M. Taboada, et al., "100-megawatt Q-switched Er:glass laser." *SPIE Proceedings* (2006).
2. Oraevsky, A.A., L.B. Da Silva, A.M. Rubenchik, M.D. Feit, M.E. Glinsky, M.D. Perry, B.M Mammini, W Small, IV, and B.C. Stuart, "Plasma Mediated Ablation of Biological Tissues with Nanosecond-to-Femtosecond Laser Pulses: Relative Role of Linear and Nonlinear Absorption." *IEEE Journal of Selected Topics in Quantum Electronics* 2(4): 801-809 (1996).
3. Cain, C. P., G. Polhamus, et al. "Porcine skin visible lesion thresholds for near-IR lasers, including modeling, at two pulse durations and wavelengths." *Journal of Biomedical Optics* 11(2) (2006).
4. Zohner J.J., K.J. Schuster , L.J. Chavey, D.J. Stolarski, S.S. Kumru, B.A. Rockwell, R.J. Thomas, and C.P. Cain, "Visible lesion thresholds and model predictions for Q-switched 1318-nm and 1540-nm laser exposures to porcine skin" *Proc. of SPIE* 6084(60840E) (2006).
5. Finney, D. J. (1971). Probit Analysis. New York, NY, Cambridge University Press.
6. Vogel, A. and V. Venugopalan, "Mechanisms of Pulsed Laser Ablation of Biological Tissues." *Chemical Reviews* 103(2): 577-644 (2003).
7. Welch, A. J. and M. Van Gemert, Thermal Response of Tissue to Optical Radiation. New York, Plenum Press (1995).
8. Willmott, P. R. and J. R. Huber, "Pulsed laser vaporization and deposition." *Reviews of Modern Physics* 72(1): 315-328 (2000).
9. Jacquier, S. L., "Role of tissue optics and pulse duration on tissue effects during high-power laser irradiation." *Applied Optics* 32(13): 2447-2454 (1993).

10. Kou, L., D. Labrie, and P. Chylek, "Refractive indices of water and ice in the 0.65-2.5 μ m spectral range," *Applied Optics* 32, 3531—3540 (1993).
11. Jindra, N. M., M.A. Figueira, L.J. Chavey, J.J. Zohner, B.A. Rockwell, "An alternative method of evaluating 1540 nm exposure laser damage using an optical tissue phantom." *Proc. of SPIE* 6084(60840I-1) (2006).
12. Pocock, G. M., J.J. Zohner, D.J. Stolarski, K. C. Buchanan, N.M. Jindra, M.A. Figueira, L.J. Chavey, M.L Imholte, R.J. Thomas, and B.A. Rockwell, "Injury thresholds for topical-cream-coated skin of hairless guinea pigs (*cavia porcellus*) in the near infrared region." *Proc. of SPIE* 6084(60840I-1) (2006).
13. Lukashev, A.V., B.I. Denker, et al., "Laser Damage of Skin by 1540 nm Er-glass Laser Radiation. Impact to Laser Safety Standards." Proceedings of SPIE 2965: 22-32 (1996).
14. Avdeev, P.S., Yu.D. Berezin, Yu.P. Guadakovskii, V.R. Muratov, A.G. Murzin, and V.A. Fromzel, "Experimental Determination of Maximum Permissible Exposure to Laser Radiation of 1.54 μ Wavelength." *Soviet Journal of Quantum Electronic* 8(137) (1978).
15. Mueller H.A., and W.J. Ham, "The Ocular Effects of Single Pulses of 10.6 μ m and 2.5-3.0 μ m Q-switched Laser Radiation." *Report to Los Alamos Scientific Laboratory, L. Division* (1976).
16. Dunskey, I. L., and D.E. Egbert, "Corneal Damage Threshold for Hydrogen Fluoride and Deuterium Fluoride Chemical Lasers." *Rep. SAM-TR-73-51* (1973).
17. Hu, X.H., Q. Fang, M.J. Cariveau, X. Pan, and G.W. Kalmus, "Mechanism Study of Porcine Skin Ablation by Nanosecond Laser Pulses at 1064, 532, 266, and 213 nm." IEEE Journal of Selected Topics in Quantum Electronics 37(3): 322-328 (2001).